The Effect of Fertilization on Growth and Mycorrhizae Numbers in 11-Year-Old Loblolly Pine Plantations

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ABSTRACT. In a field fertilization study with 11-year-old *Pinus taeda* fewer mycorrhizal root tips were found in all plots which were fertilized with N except in plots fertilized with N+P+K than were found in nontreated plots or plots fertilized with P only. Nitrogen appeared to be primarily responsible for the fewer number of mycorrhizal root tips when N and P were applied together, since P alone did not affect numbers of mycorrhizal tips. Most differences between N fertilized plots and those not receiving N fertilizer occurred during the first 6 months after fertilization. Height and diameter of *Pinus taeda* were increased by all fertilization treatments containing N but not by P alone. Concentrations of N increased in the needles of trees in plots which were fertilized with N. Numbers of mycorrhizal tips were inversely correlated with height and diameter growth. This growth was directly correlated with N concentrations in the needles. Phosphorus fertilization increased relative populations of *Cenococcum graniforme* mycorrhizae and the N+P+K treatment increased the relative population of a second mycorrhizal type. All fertilization treatments decreased the relative population of a third mycorrhizal type. The relative populations of two of the remaining eight types of mycorrhizae found were reduced by some, but not all, fertilization treatments containing either P or N. Manipulation of mycorrhizae type by fertilization may be a useful technique in plantation management. FOREST SCI. 23:37–44.

ADDITIONAL KEY WORDS. *Pinus taeda*, soil microbiology, symbiosis, ectomycorrhizae.

MYCORRHIZAE, because of their nearly ubiquitous presence in nature, are often overlooked as a factor in the growth of forest plantations. Yet mycorrhizae are known to influence nutrient uptake, resistance to drought and disease, and growth of forest trees (Bowen 1973, Marx 1973). Furthermore, certain species of mycorrhizal fungi are more effective in stimulating growth of forest trees than are other species (Mikola 1973).

Forest fertilization has become a widespread practice throughout Europe and the United States. Although many reports indicate increased growth (Richards and Wilson 1963, Wells 1970, Windsor and Reines 1973), there is considerable varia-

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TABLE 1. Characteristics of two North Carolina plantations of loblolly pine prior to application of fertilizer.

Characteristics		Plantation	
		Wood	Tillery
Physiographic province		Piedmont	Coastal Plain
Age	years	11	11
Spacing	meters	2 × 2.3	2.3 × 2.3
Site index (50 years)	meters	24.4	25.9
Soil texture		Sandy loam	Sandy loam
Drainage		Good	Poor
Depth of litter	mm	23	33
Acidity	pH	4.8	4.6
Organic matter	percent	3.1	2.9
N in pine needles[a] March 1972	percent	1.26	1.34
P in pine needles[a] March 1972	percent	.11	.11
Extractable soil minerals[b]			
P	kg/ha	6.4	3.4
K	kg/ha	88	49
Ca	kg/ha	376	328
Mg	kg/ha	57	48
Mn	kg/ha	68	17

[a] Foliage analysis values of less than 1.2 percent N and 0.10 percent P indicate a deficiency of these elements (Fowells and Krauss 1959).

[b] Extractable soil minerals were determined by the Agronomic Division, Soil Testing Laboratory, N. C. State Dept. of Agriculture, Raleigh, N. C., by diluted acid extraction (Chapman and Pratt 1961).

TABLE 2. Effect of fertilization on numbers of mycorrhizal tips and growth of loblolly pine in two 11-year-old plantations in North Carolina.

Fertilizer treatment,[a] kg/ha	Mycorrhizal tips per 100 cc soil[b]	Increase in height per tree[c]	Increase in diameter per tree[c]
	Number	*dm*	*cm*
No treatment (control)	1018	12.5	1.3
56 N	819*[d]	16.1*	1.8*
112 N	872*	16.1*	1.8*
25 P	1007	14.3	1.4
56 N 25 P	879*	17.3*	1.7*
112 N 25 P	862*	17.0*	2.1*
112 N 25 P 50 K	935	16.1*	2.0*
LSD 0.05	122	2.4	0.3

[a] Fertilizers were applied as NH_4NO_3, $Ca(H_2PO_4)_2$ and KCl in March of 1972.

[b] All values are averages of three replicate plots at each of two plantations at 10 bimonthly sampling dates from April to December during 1972 and 1973.

[c] Height growth values are averages from 3 replicate plots at two locations; 10 trees per plot. Diameter growth values are averages from 3 replicate plots at two locations; 20–25 trees per plot. Trees were measured in March 1972 and again in March 1974.

[d] * indicates difference from control at $P = 0.05$.

bility in the response of forest trees to application of fertilizers (Pritchett and Smith 1972). Little research has been directed toward the effect of forest fertilization on populations of mycorrhizal types in the field. Knowledge of the interaction among forest fertilization, mycorrhizae, and growth of forest trees may help explain variations in the responses of trees to fertilization.

The objective of this study was to relate numbers and types of ectomycorrhizae to concentrations of nitrogen (N), phosphorus (P), and potassium (K) in foliage and to growth of fertilized loblolly pine (*Pinus taeda* L.).

Materials and Methods

Numbers of Mycorrhizae and Tree Growth—1972–73.—Seven fertilizer treatments (Table 2) were applied to two 11-year-old plantations of loblolly pine in March of 1972. Characteristics of these plantations are shown in Table 1. Each treatment

was replicated three times per plantation on 0.0049 ha plots in a randomized block design.

Root samples were removed from each plot in soil cores at 2-month intervals between April and December during 1972 and 1973. Ten cylindrical soil cores 7.5 cm deep × 1.2 cm diameter (85 cc) were randomly collected from each plot and stored in plastic bags at 4° C until used. Cores were placed in 0.5 liter widemouth jars fitted with 1 mm mesh screen covers (two cores/jar). Cores were soaked in tap water overnight and rinsed vigorously under running water until roots and soil separated. The contents of the jars were swirled and poured onto a 0.147 mm sieve where roots and litter fragments were separated. Living mycorrhizae (those with intact cortical cells) were divided into discernible types described by Dominik (1959) on the basis of color and morphology. Mycorrhizal root tips were counted under a binocular microscope. Numbers of mycorrhizal tips were converted to surface area by the method of Lorio and others (1972).

Dominant trees were randomly selected within each plot and measured in March of 1972 before fertilization and the same trees were remeasured in March of 1974. Height measurements were made with a Spiegel Relaskop on 10 trees per plot in each plantation. Diameter measurements were taken with a diameter tape 1.4 m above the ground on 20–25 trees per plot in each plantation.

Tree Nutrition—1972.—In April 1973, seven 0.0041 ha plots were established in the plantation at Wood, North Carolina. Each plot received one of the fertilization treatments listed in Table 2. Samples of needles from the top portion of the crown of three trees per plot were collected at monthly intervals from April to December after fertilization. Youngest needles were sampled provided they were over 4 cm long, since at least two growth flushes occurred annually. Samples of roots less than 5 mm in diameter were also collected at monthly intervals from three trees per plot by excavating a single major lateral root from each tree. Root samples were not removed more than twice from any one tree. Needle samples were not taken from trees which had roots removed. Samples of both roots and needles were separately analyzed for total N, P, and K by the North Carolina State University Department of Soil Science. Nitrogen was determined by the standard Kjeldahl method, P was determined by the vanadate-molybdate-yellow method after tissue was treated with 7 percent $Mg(NO_3)_2 \cdot 6H_2O$ and K was determined by flame photometry (Chapman and Pratt 1961).

In April 1973, dendrometer bands were placed on three trees per treatment in plots established and fertilized in 1972 and 1973. Data on growth in circumference were taken at monthly intervals during 1973.

Results

Numbers of Mycorrhizae—1972–73.—Since results in both plantations were similar, data were combined (Tables 2,4). Fertilization with N appeared to be responsible for most of the changes in numbers of mycorrhizal tips (Table 2). On the average during the 2 years following fertilization, fertilization with 56 and 112 kg N/ha resulted in 14–20 percent fewer mycorrhizal tips than occurred in nonfertilized plots (Table 2). Most of this difference occurred during the first 6 months after fertilization when 40 percent fewer mycorrhizal tips were found in N-fertilized plots compared to nonfertilized plots (Fig. 1). Numbers of mycorrhizal tips in plots fertilized with N were not significantly different from numbers in nonfertilized plots the second year after fertilization (Fig. 1). Fertilization with 25 kg/ha P did not affect numbers of mycorrhizal tips compared to nonfertilized plots (Table 2).

Nitrogen appeared to be primarily responsible for the results obtained when N

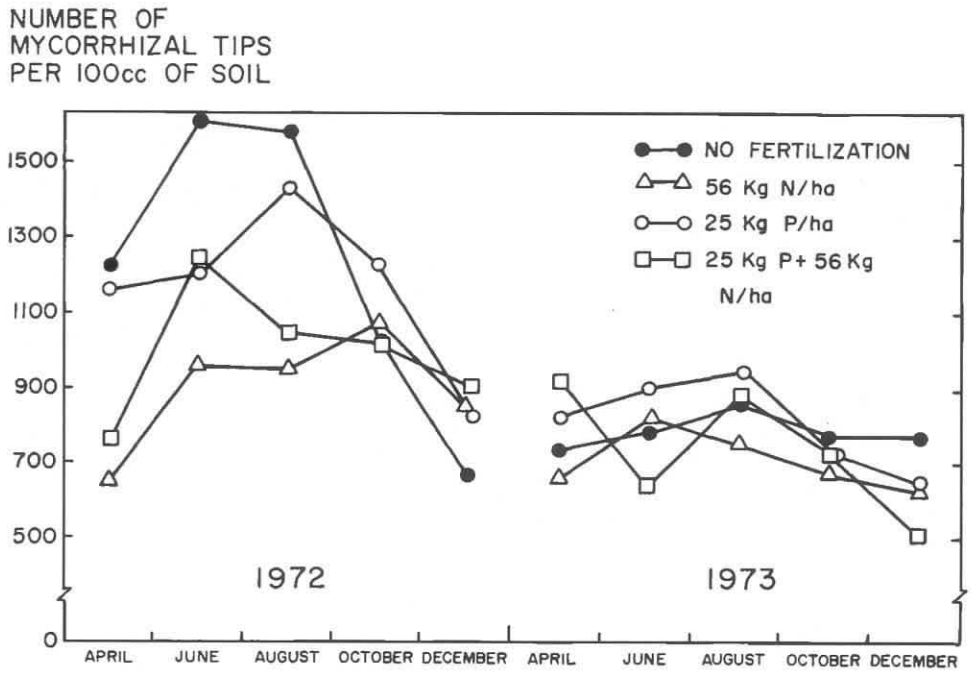

FIGURE 1. Average numbers of mycorrhizal tips during 1972–73 in plots of loblolly pine fertilized in March 1972 in North Carolina. Each point is the average from 10 soil samples per plot from three plots in each of two plantations. Variability in numbers of mycorrhizal tips was such that only during the period April–August 1972 were numbers of mycorrhizal tips significantly greater in the nonfertilized plots than in plots receiving either nitrogen alone or nitrogen plus phosphorus.

and P were applied together. Fertilization with combinations of N and P resulted in 13–15 percent fewer mycorrhizal tips, on the average during 1972 and 1973, compared to numbers in nonfertilized plots (Table 2, Fig. 1). The N+P+K fertilization did not significantly affect numbers of mycorrhizal tips (Table 2).

Tree Growth—1972–73.—Nitrogen was more effective than P in stimulating growth of loblolly pine (Table 2). Fertilization with N at 56 kg/ha and 112 kg/ha resulted in a 30 percent increase in height growth and a 32–36 percent increase in diameter growth when compared to nonfertilized trees. Fertilization with 25 kg/ha of P did not significantly affect height or diameter growth (Table 2). Combinations of N and P resulted in a 36–39 percent increase in height growth compared to nonfertilized trees. Fertilization with 56 kg N + 25 kg P/ha increased the diameter growth rate by 30 percent while 112 kg N + 25 kg/ha P increased the diameter growth rate by 61 percent compared to nonfertilized trees (Table 2). The N+P+K fertilization resulted in a 30 percent increase in height growth and a 53 percent increase in diameter growth of loblolly pine (Table 2).

Numbers of mycorrhizal tips and height growth of loblolly pine were negatively correlated ($r = -0.83$; $P = 0.05$) (Table 2). The negative correlation between diameter growth and numbers of mycorrhizal root tips was also significant ($r = -0.72$; $P = 0.05$).

Monthly diameter growth was erratic in all fertilization treatments. Greatest diameter growth occurred in August when mycorrhizal tips numbers were also at maximum.

TABLE 3. Average concentration of nitrogen, phosphorus, and potassium in the needles and roots of fertilized loblolly pines from April to December 1973.[a]

Fertilizer treatment, kg/ha[b]	N		P		K	
	Needles	Roots	Needles	Roots	Needles	Roots
No treatment	0.93	0.35	0.09	0.05	0.47	0.49
56 N	1.06*[c]	.40	.09	.05	.49	.37
112 N	1.03*	.35	.09	.06	.47	.35
25 P	.95	.34	.10*	.06	.46	.35
56 N 25 P	1.13*	.42	.10*	.08*	.49	.39
112 N 25 P	1.12*	.37	.09	.05	.53	.38
112 N 25 P 56 K	1.14*	.36	.10*	.06	.55	.40
LSD 0.05	.07	N.S.	.01	.02	N.S.	N.S.

[a] Values are averages from samples taken from three trees each month between April and December. Plots were fertilized 4 April 1973.

[b] Fertilizers were applied as NH_4NO_3, $Ca(H_2PO_4)_2$, and KCl.

[c] * indicates difference from control $P = 0.05$.

Tree Nutrition—1973.—Concentrations of N increased in the needles of trees fertilized with N (Table 3). Combinations of N and P resulted in higher concentrations of N in needles (Table 3) than occurred in needles of trees fertilized with N only. Nitrogen content of roots was not affected by any fertilizer treatments (Table 3).

Significantly more P was found in needles of trees fertilized with 25 kg P, 25 kg/P + 56 kg N/ha and the N+P+K treatment than in needles of nonfertilized trees. Phosphorus contents of the roots increased significantly only in trees fertilized with 25 kg P + 56 kg N/ha (Table 3).

Potassium contents of needles and roots were not significantly altered by fertilization.

Average concentrations of N in the needles during the first year after fertilization (1973 tree nutrition study) were inversely correlated with numbers of mycorrhizal tips the first year after fertilization (1972 mycorrhizal and tree growth study) ($r = -0.66$; $P = 0.10$). Within these two studies, a direct correlation existed between the concentrations of N in the needles the first year after fertilization (1973 tree nutrition study) and tree growth measured two years after fertilization (1972–73 mycorrhizal tree growth study) (height growth and N concentration $r = 0.90$; $P = 0.01$; diameter growth and N concentrations $r = 0.89$, $P = 0.01$).

The greatest concentrations of N and P in needles of fertilized trees were found in May, June, and July following fertilization. Differences between N and P concentrations in needles of fertilized and nonfertilized trees were also greatest during May, June, and July. The greatest difference between numbers of mycorrhizal tips in N fertilized and nonfertilized plots occurred during a similar time period (April–August) (Fig. 1).

Composition of the Mycorrhizal Population.—The relative populations of the 11 types of mycorrhizae present in the two loblolly pine plantations were substantially altered by fertilization (Table 4). There were more mycorrhizal tips of *Cenococcum graniforme* (Sow.) Ferd. & Winge ("Genus" Ga) and fewer tips of "Genus" Fi in

TABLE 4. Effect of fertilization upon the composition of mycorrhizal populations in two plantations of loblolly pine in North Carolina.

Fertilizer treatment, kg/ha[a]	Genus or type[b]											
	Ectomycorrhizae									Ectendo-mycorrhizae		Miscellaneous
	Ab	Ga	Aa	Ff	Fg	Ja	Ca	Fi	Gb	1	2	
	Percent of total mycorrhizal surface area											
No treatment	5	18	2	26	3	14	6	16	1	3	4	4
56 N	5	15	1	30	4	7**	5	16	0	3	4	8
112 N	6	17	3	30	4	6**	6	16	1	2**	4	6
25 P	6	31**[c]	2	25	3	7**	5	7**	1	2	4	7
56 N 25 P	6	26*[c]	3	30	3	5**	5	11*	0	2**	4	7
112 N 25 P	8	22	1	25	3	7**	5	15	1	2	4	8
112 N 25 P 56 K	6	20	1	35*	3	5**	5	8**	1	2**	4	10*

[a] Fertilizers were applied as NH_4NO_3, Ca $(H_2PO_4)_2$, and KCl; data for the two plantations are combined.

[b] Genera or types of mycorrhizae are those described by Dominik (1959) on the basis of color and morphology.

[c] * and ** indicate raw data (mycorrhizal surface area in mm^2) different from control at $P = 0.05$ and $P = 0.01$, respectively, before conversion to percentage.

plots fertilized with P than in nonfertilized plots. Fewer mycorrhizal tips of "Genus" Ja were found in all fertilized plots compared to nonfertilized plots. Changes in types of mycorrhizae were not correlated with tree growth or concentrations of N and P in the needles.

Discussion

Many researchers report a reduction in numbers of mycorrhizae associated with an increase in shoot growth of trees after fertilization with N (Bjorkman 1942, 1970; Dumbroff 1968; Hacskaylo and Snow 1959; Mitchell and others 1937; Richards and Wilson 1963). For moderate applications of N and P in this study this effect was relatively short-lived, lasting approximately 6 months (Fig. 1). Although most laboratory and greenhouse experiments are terminated before 6 months, Richards (1965) found that N inhibited mycorrhizae 21 weeks after fertilizer application but after 45 weeks more mycorrhizae were present on fertilized seedlings.

The relatively short-term decrease in number of mycorrhizal tips on plots fertilized with N apparently did not hinder tree growth in this study. However, fewer mycorrhizal tips occurred during the period when nutrients were most available. This could result in a reduction in the efficiency of fertilization of loblolly pine.

Nitrogen fertilization resulted in a reduction of certain types of mycorrhizae (Table 4). Other research indicates that N applications can increase numbers of mycorrhizal tips (Gobl and Platzer 1967; Koberg 1966; Meyer 1962, 1965). These results indicate that it may be possible to increase numbers of mycorrhizal tips following fertilization by inoculation with specific mycorrhizal fungi or by correctly balanced fertilization. The NPK fertilization in this study did not result in significantly fewer mycorrhizal tips than occurred in the nonfertilized plots. This result

could imply that balanced fertilization does not reduce the number of mycorrhizal tips to the degree that imbalanced fertilization does. However, the result may simply be due to the effect of K on mycorrhizal tip numbers, particularly type Ff (Table 4).

Mikola (1973) states that there is little chance of altering the types of mycorrhizae in forest plantations since the fungi best suited to the area are probably already present. However, the types best suited to N deficient sites are most likely to be adversely affected by N fertilization. This may account for fewer mycorrhizal tips in plots fertilized with N in this study. Since N additions alter the habitat for mycorrhizal fungi it may be feasible to supplant the normal mycorrhizal flora with a type better able to function despite high concentrations of N.

The types of mycorrhizae in a forest plantation can be substantially altered by applications of N, P, and K (Table 4). These results indicate that manipulation of mycorrhizae in a forest plantation by chemicals is a distinct possibility. Mycorrhizae can differ substantially in their effect upon nutrient uptake and growth by forest trees (Bowen 1973, Mikola 1973). Manipulation of mycorrhizae in forest plantations by application of chemicals or inoculation with mycorrhizal fungi could result in more efficient use of fertilizers and also increase tree growth aside from the effects of fertilizers. It is known that mycorrhizae formed by *C. graniforme* are more drought resistant than many other types of mycorrhizae (Muttiah 1972, Worley and Hacskaylo 1959). It would appear that fertilization with P could result in increased drought resistance of loblolly pine by increasing the proportion of mycorrhizae formed by *C. graniforme* (Table 4).

Although numbers of mycorrhizal tips and growth of loblolly pine were inversely correlated, they are probably not solely dependent upon one another. Since other researchers have reported increases in numbers of mycorrhizal tips together with increases in seedling growth due to fertilization it is likely that either high concentrations of N in the needles or a rapid increase of N in the needles may result in both increased growth and, frequently but not always, the formation of chemical factors which result in the formation of fewer mycorrhizal tips.

Nitrogen applied at the rate of 112 kg/ha had no greater effect on either numbers of mycorrhizal tips or tree growth than did N applied at the rate of 56 kg/ha. Data from this study indicate that the additional N was apparently not utilized by loblolly pine (Table 3). Since loblolly pine is capable of assimilating more N than is evident in Table 3 (Fowells and Krauss 1959), it is assumed that N was either immobilized by microorganisms (Bartholomew 1965) or fixed nonenzymatically (Nommik 1965) in the soil before full utilization by the trees.

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